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(54) Title: NANOPARTICLES AND MICROPARTICLES OF NON-LINEAR HYDROPHILIC-HYDROPHOBIC MULTIBLOCK COPOLYMERS

(57) Abstract

Particles are provided that are not rapidly cleared from the blood stream by the macrophages of the reticuloendothelial system, and that can be modified to achieve variable release rates or to target specific cells or organs. The particles have a core of a multiblock copolymer formed by covalently linking a multifunctional compound with one or more hydrophobic polymers and one or more hydrophilic polymers, and contain a biologically active material. The terminal hydroxyl group of the poly(alkylene glycol) can be used to covalently attach onto the surface of the particles biologically active molecules, including antibodies targeted to specific cells or organs, or molecules affecting the charge, lipophilicity or hydrophilicity of the particle. The surface of the particle can also be modified by attaching biodegradable polymers of the same structure as those forming the core of the particles. The typical size of the particles is between 180 nm and 10,000 nm, preferably between 180 nm and 240 nm, although microparticles can also be formed as described herein. The particles can include magnetic particles or radiopaque materials for diagnostic imaging, biologically active molecules to be delivered to a site, or compounds for targeting the particles. The particles have a prolonged half-life in the blood compared to particles not containing poly(alkylene glycol) moieties on the surface.

NANOPARTICLES AND MICROPARTICLES OF NON-LINEAR HYDROPHILIC-HYDROPHOBIC MULTIBLOCK COPOLYMERS

This invention is in the area of biodegradable block copolymers and nanoparticles and

5   microparticles for the controlled delivery of biologically active materials and diagnostic purposes made from the polymers.

Background of the Invention

A major challenge in the area of the

10 parenteral administration of biologically active materials is the development of a controlled delivery device that is small enough for intravenous application and which has a long circulating half-life. Biologically active

15 materials administered in such a controlled fashion into tissue or blood are expected to exhibit decreased toxic side effects compared to when the materials are injected in the form of a solution, and may reduce degradation of sensitive compounds

20 in the plasma.

A number of injectable drug delivery systems have been investigated, including microcapsules, microparticles, liposomes and emulsions. A significant obstacle to the use of these injectable

25 drug delivery materials is the rapid clearance of the materials from the blood stream by the macrophages of the reticuloendothelial system (RES). For example, polystyrene particles as small as sixty nanometers in diameter are cleared from

30 the blood within two to three minutes. By coating these particles with block copolymers based on poly(ethylene glycol) and poly(propylene glycol), their half-lives were significantly increased. L. Illum, S.S. Davis, "The organ uptake of

35 intravenously administered colloidal particles can be altered by using a non-ionic surfactant (poloxamer 338)", FEBS Lett., 167, 79 (1984).

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Liposomal drug delivery systems have been
extensively considered for the intravenous
administration of biologically active materials,
because they were expected to freely circulate in

5   the blood. It was found, however, that liposomes
are quickly cleared from the blood by uptake
through the reticuloendothelial system. The
coating of liposomes with poly(ethylene glycol)
increases their half life substantially. The

10 flexible and relatively hydrophilic PEG chains
apparently induce a stearic effect at the surface
of the liposome that reduces protein adsorption and
thus RES uptake. T.M. Allen, C. Hansen, _Biochimica_
et Biophysica Acta, 1068, 133-141 (1991); T.M.

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D.D. Lassic, et al., _Biochimica et Biophysica Acta_,
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25 _Biochimica et Biophysica Acta_, 1062, 142-148
(1991).

European Patent Application Nos. 0 520 888 A1
and 0 520 889 A1 disclose nanoparticles made from
linear block copolymer of polylactic acid and

30 poly(ethylene glycol) for the controlled
administration of biologically active materials.
The applications do not disclose how to modify the
copolymer to vary the profile of drug release or
how modifying the copolymer would affect

35 distribution and clearance of the delivery devices
in vivo. The applications also do not teach how to
prepare nanoparticles that are targeted to specific

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cells or organs, or how to prepare nanospheres that
are useful for gamma-imaging for diagnostic
purposes.

   In U.S. Serial No. 08/690,370 filed July 23,
5   1993, injectable particles are described which are
formed of a biodegradable solid core containing a
biologically active material and poly(alkylene
glycol) moieties on the surface or of block
copolymers of the poly(alkylene glycol) moieties
10   with biodegradable polymers, which exhibit
increased resistance to uptake by the
reticuloendothelial system.

   It would be desirable to have other types of
particles for the controlled delivery of materials
15   that are not rapidly cleared from the blood stream
by the macrophages of the reticuloendothelial
system, and that can be modified as necessary to
target specific cells or organs or manipulate the
rate of delivery of the material.

20   It is an object of the present invention to
provide copolymers for preparing microparticles or
nanoparticles or coatings which decrease uptake by
the reticuloendothelial system and are readily
derivatized.

25   It is another object of the present invention
to provide particles for the controlled delivery of
diagnostic and therapeutic materials that are not
rapidly cleared from the blood stream.
It is another object of the present invention to
30   provide microparticles or nanoparticles that can be
modified as necessary to target specific cells or
organs or manipulate the rate of delivery of the
material.

   It is another object of the present invention
35   to provide biodegradable microparticles or
nanoparticles that contain detectable materials for
diagnostic imaging.

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Summary of the Invention

Non-linear multiblock copolymers are prepared by covalently linking a multifunctional compound with one or more hydrophilic polymers and one or

5   more hydrophobic bioerodible polymers to form a polymer including at least three polymeric blocks. In one embodiment, one or more hydrophilic polymers, such as polyethylene glycol (PEG) chains or polysaccharide moieties, are covalently attached

10   to a multifunctional molecule such as citric acid or tartaric acid, leaving one or more active hydroxyl, carboxylic acid or other reactive functional groups available to attach the hydrophobic polymer(s). The hydrophobic polymer,

15   such as polylactic acid (PLA), polyglycolic acid (PGA), polyanhydrides, polyphosphazenes or polycaprolactone (PCL), is then covalently linked to the multifunctional compound via an appropriate reaction such as ring opening or condensation

20   polymerization. In one embodiment, the multiblock copolymers can have several short PEG chains, for example, with a molecular weight less than 1000, attached to the multifunctional compound. Ligands can be attached to one or more polymer chains to

25   achieve a variety of properties for a wide range of applications.

The block copolymers are useful in forming coatings on implantable devices and, in the most preferred embodiment, nanoparticles and

30   microparticles that are not rapidly cleared from the blood stream by the macrophages of the reticuloendothelial system, and that can be modified as necessary to achieve variable release rates or to target specific cells or organs as

35   desired. The particles can incorporate within or on their surface a substance to be delivered for either therapeutic or diagnostic purposes. In a

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preferred embodiment, the hydrophilic polymer is a
poly(alkylene glycol) (PAG). The terminal hydroxyl
group of the poly(alkylene glycol) or other
hydrophilic polymers can be used to covalently

5   attach molecules onto the surface of the particles.
Materials incorporated onto or within the particles
include biologically active molecules and targeting
molecules such as antibodies immunoreactive with
specific cells or organs, compounds specifically

10   reactive with a cell surface component, magnetic
particles, detectable materials such as radiopaque
materials for diagnostic imaging, other substances
detectable by x-ray or ultrasound such as air,
fluorescence, magnetic resonance imaging, and

15   molecules affecting the charge, lipophilicity or
hydrophilicity of the particle.
        The typical size of the particles is between
approximately 80 nm and 10,000 nm, preferably
between 80 nm and 400 nm, although microparticles

20   can also be formed as described herein. The
particles can be administered by a variety of ways,
although a preferred embodiment is by intravenous
administration. The particles are easily
lyophilized and redispersed in aqueous solutions.

25   Biodistribution experiments indicate that the
particles have a prolonged half-life in the blood
compared to particles not containing poly(alkylene
glycol) moieties on the surface.

                    Brief Description of the Figures
30   Figures 1a, 1b, and 1c are schematic
representations of nanospheres formed of multiblock
copolymers made by covalently linking a
multifunctional compound with one or more
hydrophilic polymers and one or more hydrophobic

35   bioerodible polymers.

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Figure 2a is a schematic illustration of the
synthesis of (PEG)$_3$-citrate-polylactide, (PEG)$_3$-
citrate-polycaprolactone and (PEG)$_3$-citrate-
polysebacic acid, in which the polyethylene glycol

5   blocks can be functionalized with a ligand.

Figure 2b is a schematic illustration of
multiblock copolymers of tartaric acid and mucic
acid with polylactic acid (PLA), polycaprolactone
(PCL), polysebacic acid (PSA) and polyglycolic acid

10   (PGA) hydrophobic blocks, and polyethylene glycol
(PEG) hydrophilic blocks.

Figure 2c is a schematic illustration of PEG-
di-PLA.

Figure 2d is a schematic illustration of

15   multiblock copolymers of benzene tetracarboxylic
acid with polyethylene glycol (PEG) and polylactic
acid (PLA) or polysebacic anhydride (PSA) blocks.

Figure 2e is a schematic illustration of the
synthesis of butane diglycidyl ether-based tetra-

20   arm diblock copolymers with polylactic acid (PLA)
and polyethylene glycol (PEG) blocks.

Figure 2f is a schematic illustration of
multiblock copolymers of the 1,4-3,6-dilactone of
glucaric acid with ligand, polylactic acid (PLA)

25   and polyethylene glycol (PEG) blocks.

Figure 2g is a schematic illustration of
(PEG)$_3$-citrate-polylactide in which the PEG blocks
are further functionalized with a ligand or PLA.

Figure 2h is a schematic illustration of

30   PLA-citrate-dextran and PLA-2-hydroxyadipaldehyde-
Dextran.

Figure 2i is a schematic illustration of PEG
2-hydroxyadipaldehyde-PLA in which the PEG can be
functionalized with a ligand or a methyl group.

35   The non-linear block copolymers in each of
figures 2a through 2i were synthesized from
poly(ethylene glycol) [PEG] of the molecular

weights 600, 1900, 5,000; 12,000; and 20,000, and
polylactide (PLA), polyglycolide, polycaprolactone
(PCL), or polysebacic anhydride (PSA).

5   **Detailed Description of the Invention**
     Non-linear multiblock copolymers are prepared
by covalently linking a multifunctional compound
with one or more hydrophilic polymers and one or
more hydrophobic bioerodible polymers to form a
10   polymer including at least three polymeric blocks.
In one embodiment, one or more hydrophilic
polymers, such as polyethylene glycol (PEG) chains
or polysaccharide moieties, are covalently attached
to a multifunctional molecule such as citric acid
15   or tartaric acid, leaving one or more active
hydroxyl, carboxylic acid or other reactive
functional groups available to attach the
hydrophobic polymer(s). The hydrophobic polymer,
such as polylactic acid (PLA), polyglycolic acid
20   (PGA), polyanhydrides, polyphosphazenes or
polycaprolactone (PCL), is then covalently linked
to the multifunctional compound via an appropriate
reaction such as ring opening or condensation
polymerization.
25   Particles formed of the coblock polymers are
disclosed that are not rapidly cleared from the
blood stream by the macrophages of the
reticuloendothelial system as the particles not
surface modified with hydrophilic polymers, and
30   that can be modified as necessary to achieve
variable release rates or to target specific cells
or organs as desired. The particles are useful to
administer biologically active materials in a
controlled manner for a wide variety of purposes.

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I.   Non-linear Block Copolymers.
Selection of Polymers.
Hydrophilic Polymers

Hydrophilic polymers, including but not
5   limited to poly(alkylene glycols) (which can also
be referred to as a poly(alkylene oxide), if the
polymer was prepared from an oxide instead of a
glycol) and polysaccharides, are employed as the
hydrophilic portion of the multiblock copolymer.
10   Hydrophilic polymers other than poly(alkylene
glycol) that can be used include polypyrrolidone,
poly(amino acids), including short non-toxic and
non-immunogenic proteins and peptides such as human
albumin, fibrin, gelatin and fragments thereof,
15   dextrans, and poly(vinyl alcohol). Other materials
include a Pluronic^{TM} F68 (BASF Corporation), a
copolymer of polyoxyethylene and polyoxypropylene,
which is approved by the U.S. Food and Drug
Administration (FDA).
20   As used herein, the term poly(alkylene glycol)
refers to a polymer of the formula $HO-[(alkyl)O]_y-$
OH, wherein alkyl refers to a C_1 to C_4 straight or
branched chain alkyl moiety, including but not
limited to methyl, ethyl, propyl, isopropyl, butyl,
25   and isobutyl. Y is an integer greater than 4, and
typically between 8 and 500, and more preferably
between 40 and 500.
In vivo results show that the higher the
molecular weight (MW) of PEG, the longer the
30   circulation time in the blood (the half-life).
Specific examples of poly(alkylene glycols)
include poly(ethylene glycol), polypropylene 1,2-
glycol, poly(propylene oxide) and polypropylene
1,3-glycol. A preferred hydrophilic polymeric
35   moiety is PEG of a molecular weight of between
approximately 300 to 20,000.

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To ensure elimination from the body, the
molecular weight of the polyethylene glycol should
be between approximately 300 and 20,000 Daltons,
the molecular weight of polysaccharides should be

5   40,000 or less, and the molecular weight of
proteins should be 70,000 or less.

Hydrophobic Polymers

The hydrophobic polymer should be bioerodible,
biocompatible, and have a terminal group that can

10   react with the terminal functional group, such as a
hydroxyl, thiol, amino, carboxy, aldehyde or other
functional group of the multifunctional molecule to
form a covalent linkage. Multiblock copolymers
containing polylactic acid moieties are a preferred

15   embodiment. However, the copolymer of lactic acid
and glycolic acid, as well as other polymers such
as polyanhydrides, polyphosphazenes, polymers of α-
hydroxy carboxylic acids, polyhydroxybutyric acid,
polyorthoesters, polycaprolactone, polyphosphates,

20   or copolymers prepared from the monomers of these
polymers can be used to form the multiblock
copolymers described herein. The variety of
materials that can be used to prepare the block
copolymers forming the particles significantly

25   increases the diversity of release rate and profile
of release that can be accomplished *in vivo*.

In a preferred embodiment, a polyester of
poly(lactic-co-glycolic)acid (PLGA) is used as a
hydrophobic erodible polymer bound to the

30   multifunctional compound. These polymers are
approved for parenteral administration by the FDA.
Because PLGA degrades via hydrolysis, *in vivo*
degradation rates can be predicted from *in vitro*
data. PLGA degrades to lactic and glycolic acids,

35   substances found naturally in the body.
Furthermore, by manipulating the molar ratio of

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lactic and glycolic acid and the molecular weight
of the copolymers, different degradation patterns
can be obtained.

5   The molecular weight and the chemical
composition and stereochemical configuration of the
polymer will affect the solubility of the polymer
in various organic solvents as well as the
crystallinity of the polymer. In this regard, a
copolymer of lactic acid and glycolic acid is
10   preferable.

Preferably, the hydrophobic, bioerodible
polymers are soluble in ethyl acetate or acetone.
Ethyl acetate or acetone is preferred over other
organic solvents such as dichloromethane and
15   chloroform because they are less toxic for *in vivo*
applications.

Poly L-lactide is a polymer with a high degree
of crystallinity. Poly D,L-lactide is less
crystalline and more soluble in organic solvents.
20   A random copolymer of D,L-lactide and glycolide in
the ratio of 75:25 is very soluble in organic
solvents, in particular in ethyl acetate. This
copolymer is completely amorphous, which renders it
a useful polymer for the fabrication of nanospheres
25   and microspheres for controlled release.

Poly-L-lactide has a degradation time *in vitro*
of months to years. The long degradation time is
due to its higher crystallinity which protects the
polymer from water penetration. Since D,L-lactide
30   is amorphous, its degradation time is typically one
to a number of months. Poly-glycolide also has a
crystalline structure and a degradation time of one
to several months. D,L-PLGA is amorphous, with a
degradation time *in vitro* of weeks to months. As
35   the glycolic acid ratio is increased, the rate of
degradation is enhanced. Lactic acid has bulky
methyl groups on the alpha carbon ($-O-CH(CH_3-CH-)$

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which makes it difficult for water molecules to
access the ester, while glycolic acid has a proton
on the alpha carbon ($-O-CH_2-CO-$), which allows
easier access of water molecules to the ester
5   bonds.

The molecular weight of the hydrophilic and
hydrophobic regions of the particle affect the
water solubility of the particles and thus their
stability in aqueous solutions.

10   **Preparation of multiblock copolymers.**
The multiblock copolymers formed by covalently
linking a multifunctional compound with one or more
hydrophilic polymers, preferably poly(alkylene
glycol) (PAG), more preferably poly(ethylene
15   glycol), and one or more hydrophobic polymers can
be prepared by a number of methods. One method
involves protecting one end of the hydrophilic
polymer, for example, polyethylene glycol, and
reacting the functional group at the unprotected
20   end with one or more reactive groups on the
multifunctional compound. Then, the remaining
reactive groups on the multifunctional compound can
be reacted with one or more hydrophobic bioerodible
polymers, followed by removal of the protecting
25   groups. Selective removal of the protecting groups
allows selective modification of the hydrophobic
and hydrophilic polymers, and is well known to
those skilled in the art of polymer synthesis.
Preferred protected polyalkylene glycols
30   include monomethoxy poly(alkylene glycols), such as
monomethoxy-PEG or PEG protected with another
oxygen protecting group known to those of skill in
the art, such that one terminal hydroxyl group is
protected and the other is free to react with the
35   polymer.

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A second method involves reacting a
hydrophobic bioerodible polymer, with one terminal
functional group protected, with one or more
reactive groups on the multifunctional compound,
5   and then reacting a protected hydrophilic polymer
with one or more reactive groups remaining on the
multifunctional compound.

In an alternative embodiment, a carboxylic
acid group on the multifunctional compound can be
10 reacted with a poly(alkylene glycol) terminated
with an amino function (available from Shearwater
Polymers, Inc.) to form an amide linkage, which is
in general stronger than an ester linkage. The
amide linkage may provide a longer period of
15 retention of the poly(alkylene glycol) on the
surface of the nanoparticle. Methods of linking
amino groups with carboxylic acid groups to form
amides are well known to those skilled in the art.

In another alternative embodiment, a thiol
20 group on a polymer can be reacted with a carboxy
group on the multifunctional compound to form a
thioester linkage. Methods of forming thioester
linkages are known to those skilled in the art.

In yet another alternative embodiment, amino
25 groups on a polymer can be coupled with amino
groups on a multifunctional compound using a
crosslinking agent such as glutaraldehyde. These
coupling reactions are known to those skilled in
the art.

30 Other multiblock copolymers terminated with
poly(alkylene glycol), and in particular,
poly(ethylene glycol), can be prepared using the
reactions described above, using a branched or
other suitable poly(alkylene glycol) and protecting
35 the terminal groups that are not to be reacted.
Shearwater Polymers, Inc., provides a wide variety
of poly(alkylene glycol) derivatives.

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In one embodiment, a multiblock copolymer is
prepared by reacting the terminal group of the
hydrophobic polymeric moiety such as PLA or PLGA
with a suitable polycarboxylic acid monomer,
5   including but not limited to 1,3,5-
benzenetricarboxylic acid, butane-1,1,4-
tricarboxylic acid, tricarballylic acid (propane-
1,2,3-tricarboxylic acid), and butane-1,2,3,4-
tetracarboxylic acid, wherein the carboxylic acid
10   moieties not intended for reaction are protected by
means known to those skilled in the art. The
protecting groups are then removed, and the
remaining carboxylic acid groups reacted with a
hydrophilic polymer, such as a poly(alkylene
15   glycol). In another alternative embodiment, a di,
tri, or polyamine is similarly used as the
branching agent.

II. Preparation of Particles from Block Copolymers

Preparation and Characterization of
20   **Nanoparticles**
Nanospheres can be prepared from the block
copolymers by emulsion/evaporation techniques using
the pre-formed copolymer. The pre-formed polymer
and, optionally, a substance to be delivered, if
25   soluble in an organic solvent, can be dissolved in
an organic solvent. Loadings can be about 25 mg
polymer/2 ml methylene chloride, and the substance
to be delivered in approximately between 10% and
50% of the weight of the polymer. The resulting
30   organic solution can be emulsified with an aqueous
phase by vortexing and then sonicated, typically
for 1 minute, at approximately a 40 watt output.
The solvent can be evaporated and the nanospheres
can be collected by centrifugation (30 min, 5,000
35   rpm), washed twice and lyophilized.

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Amphiphilic multiblock copolymers can form
nanospheres with a biodegradable and dense core
able to entrap drugs or other compounds, and with
an effective coating to prevent the rapid

5   recognition by the immune system. The different
solubilities of the hydrophilic and hydrophobic
blocks, for example, PEG and a polyester or
polyanhydride, in water and organic solvents allows
one to obtain the desired phase-separated structure

10   of the nanospheres. The organic phase, containing
polymer and drug, can be emulsified with water
without adding any further stabilizer, because of
the surfactant properties of the multiblock
copolymer. By emulsifying the two phases, the

15   hydrophilic block migrates to the water interface,
and the hydrophobic block remains inside the
droplets and forms the solid biodegradable core
after solvent evaporation. Sub-200 nm size
particles with a high PEG density on the surface

20   can be obtained using a high energy form such as
ultrasound. AFM analysis indicates that
nanospheres prepared in this manner are spherical,
and QELS showed that the particle size of
nanospheres prepared in this manner are in the

25   range of between 180 and 240 nm and have a unimodal
size distribution.

For example, the mixture of block copolymer
and substance to be delivered can be mixed in a
common solvent such as ethyl acetate or methylene

30   chloride. Preferably, the organic solvent is a
nonsolvent for the hydrophilic polymers, and a
solvent for the hydrophobic polymers. An emulsion
can be formed by adding water, preferably distilled
deionized water, to the solution. Slow evaporation

35   of the organic solvent allows a reorganization of
the polymer chains inside and on the surface of the
droplets. The hydrophilic polymers, which are

pref rably insoluble in the organic solvent, tend
to migrate to the aqueous phase, while the
hydrophobic polymers, which are not soluble in
water, remain inside the droplets and forms the
5   core of the nanospheres after the solvent is
evaporated. PEG chains inside the core should be
avoided, because this can lead to absorption of
water by the core followed by the accelerated and
uncontrolled release of the drugs.
10   After removing the organic solvent, the
particles can be isolated from the aqueous phase by
centrifugation. They can later be readily
redispersed in water.
         In an alternative embodiment, acetone,
15   methanol, or ethanol and their aqueous solutions
can be used in place of the distilled deionized
water. In general, water is preferred because it
forces a higher concentration of poly(alkylene
glycol) to the surface of the particle. However,
20   acetone can be used as the precipitating solvent if
the hydrophobic polymer, for example,
polyanhydride, is sensitive to water.
         In another alternative embodiment, the
multiblock copolymer can be blended with a linear
25   hydrophobic-hydrophilic copolymer, for example
PLGA-PEG mixed with PLGA or PLA, prior to
fabrication into the particles, to provide
different properties on the particles, for example,
altering their half-life *in vivo*. Adding PLGA-PEG
30   to other polymers can increase the *in vivo* half-
life of the particles.
         In a typical embodiment, the linear copolymer
can be mixed with the multiblock copolymer in a
ratio of greater than 0 up to 100 percent by weight
35   and optimally, between 10 and 100 percent by
weight.

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The substance to be delivered can be mixed
with the copolymer or copolymer blend in a ratio of
greater than 0 to 99, and more preferably, in a
ratio of 1 to 70.

5   Characterization studies were carried out at
different drug loadings to investigate
encapsulation properties and morphological
characteristics of PEG-polyanhydride and PEG-
polyester nanospheres. Particle size was measured
10 by quasi-elastic light scattering (QELS). The
instruments used were a Lexel Argon-ion laser
(Fremont, CA, USA) (model BI-200SM), with a
Brookhaven apparatus consisting of a goniometer and
a 136 channel digit correlator and a signal
15 processor. Measurements were made with a laser at
a wavelength of 488 nm at a scattering angle of
90°. The image of the nanospheres was taken by
atomic force microscopy (AFM). The apparatus
(Nanoscope III, Digital Instruments, Santa Barbara,
20 CA, USA) consisted of a cantilever oscillating
vertically (tapping mode) with a frequency of 350
kHz.

Chemical surface analysis (XPS) was performed
to check for the presence of PEG on the nanospheres
25 surface, and to investigate the presence of drug
molecules located on the surface. Data were
collected by MgKα x-rays with a power of 300 W on a
Perkin-Elmer 5100 apparatus.

To check polymer degradation, lactic acid was
30 detected by colorimetric method using Lactate
Reagent (Sigma) for a quantitative determination of
lactate at 540 nm.

Differential scanning calorimetry (DSC) was
performed to detect drug crystallization inside the
35 nanospheres and to investigate any possible
interaction between the drug and the polymer.

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Morphological analysis of the nanosphere inner core was carried out by transmission electron microscopy of a cross-section of samples obtained by freeze fracture.

5   Drug loading was measured by dissolving lyophilized nanospheres into an appropriate solvent and assaying the amount of drug (lidocaine or prednisolone) spectrophotometrically.

PEG-coated nanospheres are examples of
10   preferred nanospheres, and can be prepared from multiblock copolymers formed by covalently linking a multifunctional compound with at least one poly(ethylene glycol) (PEG) and at least one hydrophobic bioerodible polymer, such as a
15   polyester, for example, (poly(D,L lactic acid), or poly(lactic co-glycolic acid), a polylactone such as ε-polycaprolactone) or a polyanhydride, such as (poly(sebacic acid).

Light scattering studies have indicated that
20   the size of the resulting particles can be determined by the viscosity of the organic phase, ratio of organic to aqueous phase, and sonication power and time. Increased viscosity yields bigger particles and a higher ratio of the aqueous phase
25   volume as compared to organic phases yields smaller particles. An example of the effect of the sonication power and time is as follows: 25 mg polymer/2 ml CH_2Cl_2 is added to 30 ml of 0.3% polyvinyl alcohol solution. The mixture is
30   vortexed for 30 seconds at the maximum strength and then sonicated by probe sonicator for 30 seconds at the output 7. The conditions can reproducibly yield nanoparticles of a particle size of between 180 and 240 nm. These parameters can be optimized
35   to obtain nanospheres having desired size range with a narrow unimodal size distribution of about 200 nm.

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Using non-linear block copolymers, the density
of the hydrophilic block at the nanosphere surface
can be increased and blood circulation of these
carriers can be prolonged, relative to using a
5   linear copolymer. When multiblock copolymers
containing multiple PEG blocks are used, there is
typically more PEG on the surface of nanospheres
prepared from brush copolymers than on the surface
of nanospheres prepared from linear copolymers, as
10   shown by ESCA. The amount of PEG (deducted from
the ratio between PEG and PLA or PLGA comparing C
peaks convolution) can be increased from 35.65% to
more than 44% using non-linear multiblock
copolymers as compared with linear copolymers.
15   Other characterization studies were carried
out to investigate morphological characteristics
and encapsulation properties of PEG-polyanhydride
and PEG-polyester nanospheres, at different drug
loadings. Cross-section images of freeze-fractured
20   nanospheres were obtained by TEM, showing the
particle dense core. Partial drug
recrystallization was shown by DSC data.
The chemical composition of the nanosphere can
be important to the determination of the final
25   particle size. Nanospheres prepared from
multiblock brush copolymers that include a
significant amount of PEG on the surface of the
particle are typically in the size range of 180 nm
or greater. The diameter can increase up to 240 nm
30   in the case of the highest PEG m.w. in (PEG 20K)$_3$-
PLA particles, in contrast to PLA nanoparticles,
where the diameter can be less than 120 nm.
Surprisingly, this is in contrast to particles
prepared from linear copolymers, such as PEG-PLGA
35   particles, in which the PEG in PEG-PLGA particles
was able to reduce nanosphere size, as compared to
not-coated particles. The composition of the

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hydrophobic block(s) also affects the particle
size. For example, using polycaprolactone, which
is more soluble in methylene chloride, to form the
nanosphere core, particles with a diameter of less

5   than 100 nm can be obtained. Drug loading appears
to have little effect on particle size. Particles
loaded with lidocaine and prednisolone can show the
same size even when the amount of drug loaded is as
high as 45%.

10   **Preparation of Microparticles**
Microparticles can be prepared using the
methods as described above for preparing
nanoparticles, without using an ultrasonic bath.
The microparticles can also be prepared by spraying

15   a solution of the multiblock copolymer in organic
solvent into an aqueous solution.

            Composition of Particles
As described above, particles are formed from
multiblock copolymers prepared by covalently

20   linking a multifunctional compound with at least
one hydrophilic polymer, such as a poly(alkylene
glycol) with a molecular weight of between 300 and
20,000 or a polysaccharide moiety, and at least one
hydrophobic polymer, such as polyorthoesters,

25   polyphosphate esters, poly(lactic-co-glycolic
acid), poly(lactic acid), poly(glycolic acid),
polyanhydride, polyphosphazenes, polycaprolactone
or other biodegradable, biocompatible polymers, and
copolymers thereof. The multifunctional compound

30   can be substituted with between one and ten
hydrophilic polymers and between one and ten
hydrophobic polymers, and preferably is substituted
with between one and six hydrophilic polymers and
between one and six hydrophobic polymers.

20

As used herein, a hydrophilic polymer refers
to a polymer that is solubl in aqueous medium, and
if used for medical applications, is biocompatible
and readily eliminated from the human body. The
5   preferred molecular weight for PEG is between 300
and 20,000, for polysaccharides, between 1,000 and
40,000, and for polyamino acids (peptides), between
1,000 and 70,000.

As used herein, a hydrophobic bioerodible
10   polymer refers to a polymer that is insoluble in
aqueous medium, but may absorb water up to 30% of
its weight, and is biocompatible and degradable.
Preferred molecular weight ranges are between 500
and 500,000.

15   As used herein, a polysaccharide refers to a
carbohydrate composed of many monosaccharides.

As used herein, a multifunctional compound
refers to a compound with at least two functional
groups capable of being coupled with functional
20   groups on a polymer. The compound can be a linear,
branched or cyclic alkyl group, an aromatic group,
a heterocyclic group, or a combination thereof.
The types of groups include, but are not limited
to, hydroxyl, thiol, amino, carboxylic acid,
25   aldehyde, sulfonic acid, phosphoric acid, amide,
isocyanate, imine and derivatives thereof.
Preferably, the compound is non-toxic and
biodegradable. Examples of preferred
multifunctional compounds include, but are not
30   limited to, tartaric acid, mucic acid, citric acid,
glucaronic acid and tri, tetra- and polycarboxylic
acids, including benzene tetracarboxylic acid,
dextrins and tri, tetra and polyalcohols, and
molecules with combinations of carboxyl and
35   hydroxyl groups.

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Siz   of Particles

As described herein, the typical size of the
particles is between 80 nm and 10,000 nm,
preferably between 80 nm and 400 nm. The
5   methodology produces particles between 80 and
10,000 nm, i.e., both nanoparticles, and
microparticles having a diameter of 1 micron or
greater. For ease of reference herein in the
general descriptions, both microparticles and
10   nanoparticles will be referred to as particles
unless otherwise specified.

As used herein, the term nanoparticle refers
to a solid particle of size ranging from 10 to 1000
nm. The 'ideal' nanoparticle is biodegradable,
15   biocompatible, has a size of less than 200 nm and
has a rigid biodegradable core into which a
substance to be delivered can be incorporated.

The term "microparticle," as used herein,
refers to a particle of size ranging from one or
20   greater up to 1000 microns.

The nanoparticles specifically described
herein can be fabricated as microparticles if more
appropriate for the desired application.

Structure of Particles.

25   Figures 1a, 1b and 1c are schematic
representations of embodiments of a nanoparticle
prepared as described herein. Figure 1a, the
particle 10 has a biodegradable solid core 12
containing a biologically active material 14, and
30   one or more poly(alkylene glycol) moieties 16 on
the surface. The surface poly(alkylene glycol)
moieties 16 have a high affinity for water that
reduces protein adsorption onto the surface of the
particle. The recognition and uptake of the
35   nanoparticle by the reticulo-endothelial system
(RES) is therefore reduced. The terminal hydroxyl
group of the poly(alkylene glycol) can be used to

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covalently attach biologically active molecules, as
shown in Figure 1b, or molecules affecting the
charge, lipophilicity or hydrophilicity of the
particle, onto the surface of the nanoparticle.   In
5   Figure 1c, the PEG is a branched shorter chain PEG
molecule than in Figure 1a.

     A nanosphere refers to a nanoparticle that is
spherical in shape.   The shape of the nanoparticles
prepared according to the procedures herein or
10   otherwise known is easily determined by scanning
electron microscopy.   Spherically shaped
nanoparticles are preferred for circulation through
the bloodstream.   If desired, the particles can be
fabricated using known techniques into other shapes
15   that are more useful for a specific application.

     Degradation Properties.

     The term biodegradable or bioerodible, as used
herein, refers to a polymer that dissolves or
degrades within a period that is acceptable in the
20   desired application (usually *in vivo* therapy),
usually less than five years, and preferably less
than one year, on exposure to a physiological
solution with a pH between 6 and 8 having a
temperature of between 25 and 37°C.   In a preferred
25   embodiment, the nanoparticle degrades in a period
of between 1 hour and several weeks, depending on
the desired application.

     Copolymers for the Construction of Nanospheres

     The period of time of release, and kinetics of
30   release, of the substance from the nanoparticle
will vary depending on the copolymer or copolymer
mixture or blend selected to fabricate the
nanoparticle.   Given the disclosure herein, those
of ordinary skill in this art will be able to
35   select the appropriate polymer or combination of
polymers to achieve a desired effect.

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III. Substanc s to be Incorporated Onto or Into Particles

Materials to be delivered

A wide range of biologically active materials
5   or drugs can be incorporated onto or into the
particles. The substances to be incorporated
should not chemically interact with the polymer
during fabrication, or during the release process.
Additives such as inorganic salts, BSA (bovine
10 serum albumin), and inert organic compounds can be
used to alter the profile of substance release, as
known to those skilled in the art. Biologically-
labile materials, for example, procaryotic or
eucaryotic cells, such as bacteria, yeast, or
15 mammalian cells, including human cells, or
components thereof, such as cell walls, or
conjugates of cellular can also be included in the
particle. The term biologically active material
refers to a peptide, protein, carbohydrate, nucleic
20 acid, lipid, polysaccharide or combinations
thereof, or synthetic inorganic or organic
molecule, that causes a biological effect when
administered *in vivo* to an animal, including but
not limited to birds and mammals, including humans.
25 Nonlimiting examples are antigens, enzymes,
hormones, receptors, and peptides. Examples of
other molecules that can be incorporated include
nucleosides, nucleotides, antisense, vitamins,
minerals, and steroids.
30   Particles prepared according to this process
can be used to deliver drugs such as nonsteroidal
anti-inflammatory compounds, anesthetics,
chemotherapeutic agents, immunotoxins,
immunosuppressive agents, steroids, antibiotics,
35 antivirals, antifungals, and steroidal
antiinflammatories, anticoagulants. For example,
hydrophobic drugs such as lidocaine or tetracaine

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can be entrapped into the particles and are
released over several hours. Loadings in the
nanoparticles as high as 40% (by weight) have been
achieved. Hydrophobic materials are more difficult
5   to encapsulate, and in general, the loading
efficiency is decreased over that of a hydrophilic
material.

In one embodiment, an antigen is incorporated
into the nanoparticle. The term antigen includes
10   any chemical structure that stimulates the
formation of antibody or elicits a cell-mediated
humoral response, including but not limited to
protein, polysaccharide, nucleoprotein,
lipoprotein, synthetic polypeptide, or a small
15   molecule (hapten) linked to a protein carrier. The
antigen can be administered together with an
adjuvant as desired. Examples of suitable
adjuvants include synthetic glycopeptide, muramyl
dipeptide. Other adjuvants include killed
20   *Bordetella pertussis*, the liposaccharide of Gram-
negative bacteria, and large polymeric anions such
as dextran sulfate. A polymer, such as a
polyelectrolyte, can also be selected for
fabrication of the nanoparticle that provides
25   adjuvant activity.

Specific antigens that can be loaded into the
nanoparticles described herein include, but are not
limited to, attenuated or killed viruses, toxoids,
polysaccharides, cell wall and surface or coat
30   proteins of viruses and bacteria. These can also
be used in combination with conjugates, adjuvants,
or other antigens. For example, *Haemophilius
influenzae* in the form of purified capsular
polysaccharide (Hib) can be used alone or as a
35   conjugate with diphtheria toxoid. Examples of
organisms from which these antigens are derived
include poliovirus, rotavirus, hepatitis A, B, and

25

C, influenza, rabies, HIV, measles, mumps, rubella,
*Bordetella pertussus, Streptococcus pneumoniae, C.
diphtheria, C. tetani, Cholera, Salmonella,
Neisseria,* and *Shigella.*

5   Non-pharmaceutical uses for the particles
include delivery of food additives, including
stabilizers and dispersants or other viscosity
modifying agents, controlled and selective delivery
of pesticides, herbicides, insecticides,

10   fertilizer, and pheromones, and in color and ink
formulations in the printing and ink industry.

**Incorporation of Substances for Diagnostic
Purposes.**

15   In another embodiment, a gamma-labelled
nanoparticle is provided that can be used to
monitor the biodistribution of the particle *in
vivo.* Any pharmaceutically acceptable gamma-
emitting moiety can be used, including but not

20   limited to indium and technetium. The magnetic
particles can be prepared as described herein, or
alternatively, magnetic nanoparticles, including
surface-modified magnetic nanoparticles can be
purchased commercially, the surface further

25   modified by attaching the hydrophilic polymeric
coating.
        For example, the magnetic nanoparticle can be
mixed with a solution of the hydrophilic polymer in
a manner that allows the covalent binding of the

30   hydrophilic polymer to the nanoparticle.
Alternatively, a gamma-emitting magnetic moiety is
covalently attached to the hydrophilic or
hydrophobic bioerodible polymeric material of the
particle. The larger the size of the magnetic

35   moiety, the larger the size of the resulting
particles obtained.

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Other materials can also be incorporated into the particles for diagnostic purposes, including radiopaque materials such as air or barium and fluorescent compounds. Hydrophobic fluorescent

5   compounds such as rhodamine can be incorporated into the core of the particles. Hydrophilic fluorescent compounds can also be incorporated, however, the efficiency of encapsulation is smaller, because of the decreased compatibility of

10   the hydrophobic biodegradable core with the hydrophilic material. The hydrophilic material must be dissolved separately in water and a multiple emulsion technique used for fabrication of the particle.

15   In one embodiment, the particles include a substance to be delivered and a multiblock copolymer that is covalently bound to a biologically active molecule, for example, an antibody or antibody fragment, such as the Fab or

20   Fab$_2$ antibody fragments, wherein the particle is prepared in such a manner that the biologically active molecule is on the outside surface of the particle.

         Modification of Surface Properties of
25   **Particles.**
         The charge, lipophilicity or hydrophilicity of the particle can be modified by attaching an appropriate compound to the hydrophilic polymer on the surface of the particle. The particle can also

30   be coated with a dextran, which are in general more hydrophilic than poly(alkylene glycol) but less flexible. Dextran coated nanoparticles are useful for magnetic resonance imaging (MRI).

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Attachment of Specific Ligands to Particle Surfaces.

The particles prepared as described herein can
be used for cell separation, or can be targeted to
5   specific tissues, by attaching to the surface of
the particle specific ligands for given cells in a
mixture of cells. When magnetic particles are also
incorporated, the particles can be targeted using
the ligands, such as tissue specific receptors or
10   antibodies to tissue specific surface proteins,
then maintained at the targeted cells using a
magnetic field while the particles are imaged or a
compound to be delivered is released.

For example, in one embodiment, carmustine
15   (BCNU) or other anti-cancer agent such as cis-
platin is incorporated in the core of the particles
and antibodies to the target cancerous cells are
covalently bound to the surface of the particle.

Pharmaceutical Administration of Nanospheres

20   The particles described herein can be
administered to a patient in a variety of routes,
for example, orally, parenterally, intravenously,
intradermally, subcutaneously, or topically, in
liquid, cream, gel or solid form.

25   The particles can be lyophilized and then
formulated into an aqueous suspension in a range of
microgram/ml to 100 mg/ml prior to use.
Alternatively, the particles can be formulated into
a paste, ointment, cream, or gel, or transdermal
30   patch.

The nanoparticle should contain the substance
to be delivered in an amount sufficient to deliver
to a patient a therapeutically effective amount of
compound, without causing serious toxic effects in
35   the patient treated. The desired concentration of
active compound in the nanoparticle will depend on
absorption, inactivation, and excretion rates of

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the drug as well as the delivery rate of the
compound from the nanoparticle. It is to be noted
that dosage values will also vary with the severity
of the condition to be alleviated. It is to be
5   further understood that for any particular subject,
specific dosage regimens should be adjusted over
time according to the individual need and the
professional judgment of the person administering
or supervising the administration of the
10   compositions.

The particles can be administered once, or may
be divided into a number of smaller doses to be
administered at varying intervals of time,
depending on the release rate of the particle, and
15   the desired dosage.

IV. Coatings of Implantable Devices

Polymers loaded as described herein can also
be used to coat implantable devices, such as
stents, catheters, artificial vascular grafts, and
20   pacemakers. The device can be coated with the
lyophilized powder of the particles, or otherwise
as known to those skilled in the art. The coating
can release antibiotics, anti-inflammatories, or
anti-clotting agents at a predetermined rate, to
25   prevent complications related to the implanted
devices. Controlled delivery devices prepared as
described herein can also be used as ocular inserts
for extended release of drugs to the eye.

Examples.

30   The preparation of specific multiblock
copolymers of hydrophobic bioerodible polymers such
as PLA and PLGA, and hydrophilic polyalkylene
glycols such as PEG, with multifunctional compounds
such as tartaric acid, mucic acid, citric acid,
35   benzene tetracarboxylic acid, glucaronic acid, and

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butane diglycidyl ether are described in detail
below. These polymers were prepared with PEG of
various chain lengths, and with various hydrophobic
polymers. Given this detailed description, one of

5   skill in the art will know how to produce a wide
variety of multiblock copolymers suitable for
fabrication into nanospheres.

Materials and Methods.

Low toxicity stannous octoate was purchased

10   from ICN. D,L-lactide was purchased from Aldrich
Chemical Company, and glycolide from Polysciences,
Inc. These compounds were recrystallized before
use from ethyl acetate. High purity monomethoxy
PEG (M-PEG) with molecular weight 5,000, 12,000 and

15   20,000 was purchased from Shearwater Polymers, Inc.
The number average molecular weight of the polymer
was determined with on a Perkin-Elmer GPC system
with an LC-25 refractive index detector equipped
with a mixed bed Phenogel column filled with 5 μm

20   particles from Phenomenex. Chloroform was used as
the eluent, with a flow rate of 0.9 ml/min. The
molecular weights were determined relative to
narrow molecular weight polystyrene and
poly(ethylene glycol) standards from Polysciences.

25   Thermal transition data was collected with a
Perkin-Elmer DSC-7 (Newton Center, MA). The sample
weight ranged from 20 to 25 mg. Indium was used
for temperature and enthalpy calibrations. Each
sample was subjected to a heat-cool-heat cycle from

30   -60 to 150°C with a rate of 10°C/min. Wide angle
x-ray diffraction spectra were obtained with a
Rigaku Rotaflex Diffractometer from Rigaku
Corporation (Danvers, MA) with S=0.05 using a
Nickel filtered Cu Kα source. The data was

35   analyzed on a Micro Vax II computer. The IR
spectra were recorded on a Nicolet 500 spectrometer
using a polymer powder melted on sodium chloride

30

crystals to obtain thin films. ^{13}C NMR studies were
conducted on samples dissolved in deuterated
chloroform with a Nicolet NT-360 spectrometer.
Peak fitting was carried out with a VG data system.

5   **Example 1:   Synthesis of (methoxy-PEG-NH$_2$)$_3$,
                     citrate (compound A)**

Three PEG citrates were prepared as follows:
PEG-NH$_2$ (1gram, MW= 5,000, Sherewater) was
reacted with citric acid (14 mg, 0.33 equivalents)
10   using dicyclohexylcarbodiimide (DCC) (54mg, 1
equivalent) and DMAP (4mg, catalyst) in 10 ml of
dry dichloromethane. The reaction was continued
for 2 days at room temperature with magnetic
stirring. The DCU by-product was isolated by
15   filtration and the filtrate was poured into 100 ml
of ether:petroleum ether 1:1 mixture. The
precipitated polymer was washed with ether and
dried to yield 0.8 grams of a white powder. The
product did not contain acid groups (Bromophenol
20   test) and showed a single peak at the GPC
chromatogram in the area of 15,000. IR showed
typical ester peak (1720 cm^{-1}). Methoxy-PEG citrate
trimers with PEG of the following molecular
weights, 1,900; 12,000; and 20,000 were prepared
25   using this procedure.

The PEG derivatives of tartaric acid
[(methoxy-PEG)2-tartrate], mucic acid [(methoxy-
PEG)-2-mucoate], and glucaronic acid
(methoxy-PEG-mucoate) with various PEG chain length
30   were prepared similarly. All derivatives possessed
the appropriate molecular weight (determined by GPC
using PEG standards), showed a negative result in
the bromophenol test for carboxylic acids, and had
an absorption peak at 1720 typical for amide bonds.

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Example 2:   **Esterification r action between methoxy PEG-OH and citric acid using DCC.**

The reaction conditions were the same as

5   above, and an 80% conversion was obtained, as determined by GPC (compound A-1)

Example 3.   **Direct esterification reaction between methoxy PEG-OH and citric acid.**

10   In a 100 ml round bottom flask equipped with a Dean-Stark azeotrope apparatus, methoxy PEG-OH (MW 1900, Polysciences) was reacted with citric acid (0.33 equivalents) in toluene and sulfuric acid as catalyst (1%). The reaction was conducted under

15   reflux using azeotrope for H_2O removal. About 75% yield was obtained as determined by GPC.

Example 4.   **Trans esterification reaction between methoxy PEG-OH and methyl citrate ester.**

20   Citrate methyl ester was obtained from the reaction between citric acid and access methanol at reflux.

The resulting trimethyl citrate (1 equivalent) was reacted with methoxy PEG Mw-1900 (3

25   equivalents) in refluxing toluene for three hours. The product was isolated in about 70% yield, as determined by GPC, after evaporation of the toluene and extraction with diethyl ether.

Example 5.   **Synthesis of**
30   **(PEG)$_3$-citrate-polylactide [PEG$_3$-PLA] or PEG$_3$-caprolactone [PEG$_3$-PCL] diblock copolymers (compound A1, Figure 2a)**

PEG$_3$-citrate (1 gram) (Sherewater, MW-5,000,

35   12,000, and 20,000) was dissolved in 20 ml benzene. Lactide (5 grams) (Aldrich, 99%+) was added and the solution was allowed to reflux and azeotrope for 60 min. Stannous octoate (0.2% by weight (per

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lactide)) was added as a 1% solution in benzene.
The reaction was refluxed for 5 hours, the solvent
was removed azeotropically and a viscous material
was obtained. The polymerization was continued for
5 2 hours at 130°C. The resulting polymer was a
clear, slightly yellow mass, and showed a high
molecular weight (Table 1). The multiblock
copolymers of PEG-polycaprolactone were similarly
synthesized. The polymers were soluble in common
10 organic solvents.

Table 1: Molecular weights of PEG block copolymers

Polymer	MP	Mn	Mw
PEG-PCL block copolymers			
PCL-PEG 5k (1:5 w/w)	29,500	70,100	55-58
PCL-PEG 12k (1:5 w/w)	25,500	88,100	55-58
PCL-PEG 20k (1:5 w/w)	34,500	105,900	50-56
PEG-citrate-PLA multiblock copolymers			
(PEG-NH 5k)$_3$ citrate	15,600	—	
(PEG-NH 5k)$_3$ citrate-PLA	71,900	228,100	65-75
(PEG-NH 5k)$_3$ citrate-PCL	42,000	170,000	52-58
PSA-PEG block copolymers			
PSA-COO-PEG 5k (3:1 w/w)	19,500	119,100	65-78
PSA-COO-PEG 12k (3:1 w/w)	21,000	144,200	65-78
PSA-COO-PEG 20k (3:1 w/w)	18,500	105,000	65-78
P(SA-PEG 5k) random block (1:3)	21,000	105,400	64-73
PSA	17,200	81,300	80-82
P(FAD)-COO-PEG 5k (3:1)	12,000	34,000	42-48
P(CPP-SA)1:1-COO-PEG 5k (3:1)	12,000	34,000	42-48

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Example 6:   Synth sis of multiblock (brush)
                PEG-ligand-PLA
                (compound B, Figur 2a)

Citric acid (0.1 mole) was reacted with a

5   mixture of methoxy-PEG amine (MW 1900) (0.2 mole)
and benzyl ester carboxy-PEG-amine (MW 5,000) (0.1
mole) using DCC (0.33 equivalents) and DMAP (0.01
mole, catalyst) in 100 ml of dry dichloromethane.
The reaction was continued for 2 days at room

10   temperature with magnetic stirring. The DCU
by-product was isolated by filtration and the
filtrate was poured into 500 ml of ether:petroleum
ether 1:1 mixture. The precipitated polymer was
washed with ether and dried to yield a white powder

15   in 90% yield. The product did not contain acid
groups (Bromophenol test) and showed a single peak
at the GPC chromatogram with a molecular weight of
9,000. IR showed typical ester peak (1720 cm^{-1}).
Block copolymers with lactide and caprolactone were

20   synthesized using the same method described for
PLA-PEG brush block copolymers.

The PLA-PEG citrate trimer was dissolved in
tetrahydrofuran and hydrogenated with
Hydrogen-Palladium catalysis to remove the benzylic

25   protecting group at the PEG 5000 chain. The end
chain carboxylic acid PEG was then reacted with
bovine serum albumin (representing a ligand) using
DCC as an activating agent for amide coupling.

Similarly, two or three ligands can be

30   attached to (PEG)3-citrate by using two or three
equivalents of benzyl carboxylate-terminated
PEG-amines, using the above method.

Example 7:   Preparation of PEG_2-tartrate-PLA_2
                (Compound C, Figure 2b)

35   Di-PEG tartrate was prepared from the reaction
between amino terminated methoxy PEG and tartaric
acid with DCC as the activating agent, using the
procedure described for the synthesis of

35

(PEG)$_3$-citrate. The di-PEG tartrate derivative was reacted with lactide or glycolide mixtures to form clear polymers (Table 1).

Example 8:   **Preparation of di-methoxy PEG-mucoate-tetra PLA (Compound D, figure 2b)**

Mucic acid (Aldrich) was reacted with two equivalents of methoxy PEG in the presence of DCC in DMF to form di-PEG-mucoate which was copolymerized with lactide, glycolide or caprolactone to form high molecular weight (Mw=65,000-95,000) hexa-armed block copolymers.

Example 9:   **Preparation of penta-methoxy PEG-glucoronate-anhydride (Compound E, Figure 2b)**

Glucaronic acid was reacted with carboxylic acid terminated methoxy PEG (MW=5,000, Sherewater) in the presence of DCC to form (PEG)$_5$-gluconate. The penta-PEG compound was polymerized with sebacic acid (1:5 weight ratio) using acetic anhydride as a dehydrating agent. Polymers with a molecular weight of approximately 75,000 were obtained.

Example 10:   **Preparation of mono-PEG-penta PLA glucoronate (Compound F, Figure 2b)**

Glucaronic acid was reacted with amino terminated methoxy PEG (MW=5,000, Sherewater) in · the presence of DCC in dichloromethane of DMF to form PEG-gluconate amide. The gluconate PEG derivative was polymerized with lactide, glycolide or caprolactone (1:5 weight ratio).

Example 11:   **Preparation of PEG-di-PLA (Compound G, Figure 2c)**

Methoxy-PEG-epoxide terminated (Sherewater) was hydrolyzed in a sodium carbonate solution overnight at room temperature. The resulted PEG

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with two hydroxyl groups was isolated by
precipitation in ether:methanol 1:1 mixture and
dried. The dihydroxy-terminated PEG was block
copolymerized with lactide, glycolide and
5   caprolactone to form high molecular weight polymers
(The molecular weight was in the range of 70,000 to
115,000).

Example 12:   **Preparation of trimethoxy**
PEG-citrate-poly(sebacic
10   **anhydride) diblock copolymer**
(Compound H, Figure 2a)

Trimethoxy-PEG-citrate (0.01 mole, prepared as
above) reacted with access adipoyl chloride (0.012
mole) in dichloromethane with triethylamine as a
15   proton acceptor. After 24 hours at room
temperature, water was added, the reaction mixture
was stirred at room temperature for one hour, and
the polymer was isolated by the adding a mixture of
methanol-diethyl ether 1:1. The resulting
20   trimethoxy-PEG-citrate-adipate was reacted with
acetic anhydride to form the acetate anhydride
derivative, which was polymerized with a sebacic
anhydride prepolymer to form a multiblock copolymer
with a molecular weight of $Mw=58,000$; $Mn=31,000$.
25   $MP=65-74°C$.

Example 13:   **Benzene tetracarboxylic anhydride**
(BTCA) derivatives
(Compound I, Figure 2d)

BTCA was reacted with two equivalents of
30   methoxy PEG amine in refluxing THF for 5 hours to
yield dimethoxy-PEG tetracarboxybenzoate, with two
remaining carboxylic groups. The PEG-dimer was
reacted with acetic anhydride and then with sebacic
anhydride to form the tetra-armed diblock
35   PEG_2-benzene-PSA_2.

Alternatively, polycaprolactone diol
(Mw=3,000, Polysciences) was reacted with

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dimethoxy-PEG tetracarboxybenzoate containing 2
carboxylic acids to form the tetra-armed PEG-PCL
diblock copolymer. The PLA or PCL block copolymers
were prepared, and then the carboxylic acid groups

5   of the PEG-benzene tetracarboxylate were reacted
with propylene oxide to form the hydroxyl
derivative available for the block copolymerization
with lactide, glycolide and caprolactone.

Example 14:   Butane diglycidyl ether based tetra-
10   **Arm   Diblock copolymers**
                (Compound J, Figure 2e)

     Butane diglycidyl ether was reacted with two
equivalents of methoxy-PEG-OH in refluxing THF for
10 hours. The PEG dimer was block copolymerized

15   with lactide, glycolide or caprolactone in toluene
with stannous octoate as catalyst. High molecular
weight polymers were obtained **(Please define high**
molecular weight).

     Example 15:   Multiblock copolymers based on the
20   **1,4;3,6-dilactone of glucaric**
                acid (Compound K, Figure 2f)

     PLA was polymerized in the presence of the
dilactone (5:1 weight ratio) using stannous octoate
as catalyst in benzene. The two carboxylic acid

25   groups were used to attach methoxy-PEG-amine via an
amide bond.

     Example 16:   Synthesis of PLA-citrate-dextran
                (Compound N, Figure 2h)

     Dextran, a clinically used biodegradable
30   material, was used as alternative hydrophilic
polymer to PEG. The benzyl ester of citric acid
was polymerized with lactide to form a PLA-
terminated citrate ester which was hydrogenated to
remove the benzyl groups. The citric acid

35   terminated-PLA was esterified with dextran to form
PLA-citrate-Dextran;

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Example 17:   Derivatives of PEG
               2-hydroxyadipaldehyde
               (Compound M, Figure 2i)

     2-Hydroxyadipaldehyde (Aldrich) was reacted

5   with amino terminated PEG to form the Schiff base
    which was hydrogenated with NaBH$_4$ to form the
    corresponding amine. The di-PEG derivative was
    reacted with lactide or caprolactone in the
    presence of stannous octoate to form the PLA or

10 PCL-PEG$_2$ diblock copolymer.

     Example 18:   Derivatives of Dextran or Ligand
                    2-hydroxyadipaldehyde
                    (Compound M-1, Figure 2h)

     2-Hydroxyadipaldehyde is reacted with lactide

15 in the presence of stannous octoate to form
    adipaldehyde-terminated PLA. The aldehyde groups
    are reacted with animo side groups of a ligand
    (peptide or protein) to form a di-ligand-PLA
    diblock. Alternatively, the aldehydic terminals

20 are reacted with ethylene diamine to form
    PLA-terminated with diamino groups. This polymer
    is reacted with an oxidized polysaccharide, such as
    dextran or amylose, to form a PLA-di-
    (polysaccharide) derivative.

25 Example 19:   Polyanhydride-PEG

     Polyanhydride-terminated PEG was prepared by
    melt condensing a sebacic acid prepolymer
    (synthesized by refluxing sebacic acid in acetic
    anhydride and precipitating the resulting polymer

30 in ether/petroleum ether solution) and methoxy PEG-
    OH or methoxy PEG-carboxylate acetate anhydride.
    In a typical experiment, methoxy-PEG-carboxylate (1
    gram) was mixed with sebacic acid prepolymer (3
    grams). The mixture was polymerized at 180°C under

35 vacuum (0.1 mm Hg) for 90 minutes to yield the
    polymer. The polymer showed IR absorption at 1805
    and 1740 cm-1 (typical for aliphatic anhydride

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bonds), and the ^{1}H-NMR spectrum fit the polymer
structure.

**Example 20:   Preparation of Nanoparticles from
Mixtures of Non-Linear Multiblock
Copolymers and Linear Polymers and
                    Copolymers**

        Nanospheres were prepared from a mixture of
PEG$_3$-citrate-PLA, a PLGA-PEG copolymer and a
polycaprolactone homopolymer in a ratio of 1:1:3 by
weight, using an emulsion/evaporation technique as
described above. The pre-formed polymers were
dissolved in an organic solvent **(Which solvent)** at
a concentration of **(What concentration?)**
polymer/solvent. The resulting organic solution
was emulsified with an aqueous phase by vortexing
and then sonicated for 1 minute at 40-W output.
The solvent was evaporated and the nanospheres were
collected by centrifugation (30 min, 5,000 rpm),
washed twice and lyophilized, yielding nanospheres
with an average size of approximately 200 nm.

Example 21:   Drug Release Characteristics

        Lidocaine and prednisolone (Sigma), were
selected for encapsulation because of their low
water solubility (less than 5 mg/mL in water), high
solubility in organic solvents (more than 20 mg/mL
in organic solvents such as chlorinated
hydrocarbons, tetrahydrofuran, dimethyl formamide
or dioxane) and ease of detection by UV
spectrophotometry.

        Release tests were carried out with
nanospheres loaded with lidocaine in different
amounts (20% wt, 33% wt), in phosphate buffer
solution (PBS, pH 7.4) at 37°C. A dialysis
membrane (50,000 cut-off) was filled with a
suspension of lyophilized nanospheres (10 mg/5 ml
PBS) and then placed into 25 ml of PBS. Samples

40

were taken from the outer solution, then replaced
every time with fresh ones. Drug released was
detected spectrophotometrically at 240 nm.

5   While high encapsulation efficiency can be
achieved with particles made from multiblock brush
copolymers, it can be difficult to obtain 100%
encapsulation efficiency due to the hydrophilicity
of the multiblock copolymers. It was observed that
the encapsulation efficiency can be less than 70%
10   for PEG_3-citrate-PLA multiblock copolymers with a
PEG (m.w. of 5, 12, 20 kDa).

        In vitro studies were performed to investigate
the release characteristics of PEG-coated
nanospheres, in particular to study the effect of
15   the presence of PEG on the nanosphere surface and
the effect of the nanosphere core composition
(polymer and drug nature, drug loading) on the drug
release kinetics. Suspensions of nanospheres were
easily obtained by redispersing freeze-dried
20   particles in aqueous solutions by vortexing,
without any further additives. Lidocaine was used
as a model drug. The release of lidocaine was
studied in particles made from linear PEG-PGLA
copolymers as well as non-linear brush copolymers.

25   Both types of particles show a continuous
release *in vitro* over several hours, but have
different release kinetics. The molecular weight
does not effect the release pattern of PEG-PLGA
nanospheres, since the drug is completely released
30   in about ten hours using copolymers with a PEG m.w.
of 5, 12, 20 KDa. The presence of PEG on the
surface of the nanospheres is not expected to
modify the drug release. However, with multiblock
copolymers, factors such as higher PEG density and
35   PEG chain length can slow down drug release. In
ten hours, more than 90% of lidocaine was released

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from PLA nanospheres, but only 60% from (PEG 20K)$_3$-PLA particles.

Drug release from nanospheres made from PEG-ϵ-polycaprolactone is biphasic.

5   Because of polymer erosion, it would ordinarily be expected that a core made of polyanhydride should lead to a faster drug release. However, after an initial fast release in the first two hours, drug release reached a plateau, although

10   drug was released at a constant rate for an additional eight hours.

Polymer degradation kinetics were also investigated *in vitro*. With PEG-PLGA, PEG-PCL and (PEG)$_3$-PLA particles, the polymers start to degrade

15   after weeks. Nanosphere cores made of polyanhydrides start to degrade immediately. In the first case, drug release is governed by a diffusion mechanism, since the drug can be completely released before polymer degradation

20   occurs. With polyanhydrides, polymer erosion affects drug release, and drug characteristics have a more important role in release kinetics. The particle's small size and large surface area increases the rate of polymer erosion relative to

25   other drug delivery systems, such as slabs, and afterwards drug solubility governs the dissolution kinetics.

The amount of drug loading can have a strong effect on the release kinetics. PEG-PLGA

30   nanospheres containing 33% wt of lidocaine can release the drug for over 12 hours. Surprisingly, particles loaded with 10% of the drug can show complete drug release in 6 hours. Increased drug loading can cause part of the drug loaded in the

35   core to recrystallize, as shown by DSC. The presence of crystals of a hydrophobic drug, such as lidocaine, can slow down the release kinetics.

ESCA studies performed on drug loaded nanospheres
confirmed that drug crystals were not located on
the nanosphere surface. The polymer composition
was also modified and the drug loading was

5   increased up to 45% wt.

**Example 22:   Evaluation of Biodistribution of
^{111}In-labeled Nanoparticles in vivo.**

Indium 111 ("In") can be directly attached to
the multiblock copolymer chains by complex

10   formation. In and diethyltriamiopentaacetic acid
(DTPA) are reacted with stearylamine. The
resulting compound, In-DTPA-stearylamide, is
hydrophobic enough to interact to be encapsulated
within the hydrophobic core. In this case, the

15   molecular weights of the hydrophilic and
hydrophobic polymers have little effect on the
interaction. After incubation at 37°C in PBS or
horse serum for more than 24 hours, label loss can
be assessed by measuring the radioactivity of the

20   supernatant solutions after centrifugation. This
labelling method can therefore be useful for in
vivo studies, by gamma-scintography or by direct
measurement of the radioactivity in the blood
and/or different organs.

25   This invention has been described with
reference to its preferred embodiments. Variations
and modifications of the invention will be obvious
to those skilled in the art from the foregoing
detailed description of the invention. It is

30   intended that all of these variations and
modifications be included within the scope of the
appended claims.

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We claim:

1. Multiblock copolymers comprising a multifunctional compound covalently linked with one or more hydrophilic polymers and one or more hydrophobic bioerodible polymers and including at least three polymer blocks.

2. The multiblock copolymer of claim 1 wherein the multifunctional compound is selected from the group consisting of dextrins, pentaerythritol, glucaronic acid, tartaric acid, mucic acid, citric acid, benzene dicarboxylic acid, benzene tricarboxylic acid, benzene tetracarboxylic acid and butane diglycidyl ether.

3. The multiblock copolymer of claim 1 wherein the hydrophilic polymer is selected from the group consisting of polyalkylene glycols, polyvinyl alcohols, polypyrrolidones, poly(amino acids), oxidized cellulose and dextrans.

4. The multiblock copolymer of claim 3 wherein the poly(amino acids) are selected from the group consisting of gelatin, fibrinogen and albumin fragments.

5. The multiblock copolymer of claim 1 wherein the hydrophobic polymer is selected from the group consisting of polyphosphazenes, polyphosphate esters, polyanhydrides, polyhydroxybutyric acid, polyorthoesters, polycaprolactone, poly(α-hydroxy acids), polybutylene glycol and copolymers prepared from the monomers of these polymers.

6. A particle having a diameter of between 50 nm and 1000 μm formed of or coated with a multiblock copolymer formed by covalently linking a multifunctional compound with one or more hydrophilic polymers and one or more hydrophobic bioerodible polymers to form a coblock polymer including at least three polymer blocks.

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7. The particle of claim 6 further comprising
a substance to be delivered selected from the group
consisting of peptides, proteins, carbohydrates,
nucleic acids, lipids, polysaccharides,
combinations thereof, and synthetic inorganic or
organic molecules that cause a biological effect
when administered to an animal.

8. The particle of claim 6 wherein the
hydrophilic polymer is selected from the group
consisting of polyalkylene glycols, polyvinyl
alcohols, polypyrrolidones, poly(amino acids),
oxidized cellulose and dextrans.

9. The particle of claim 8 wherein the
poly(amino acid) is selected from the group
consisting of gelatin, fibrinogen and albumin
fragments.

10. The particle of claim 8 wherein the
polyalkylene glycol is selected from the group
consisting of polybutylene glycol, polyethylene
glycol and copolymers of polyoxyethylene and
polyoxypropylene.

11. The particle of claim 6 wherein the
hydrophobic polymer is selected from the group
consisting of polyphosphazenes, polyphosphate
esters, polyanhydrides, polyhydroxybutyric acid,
polyorthoesters, polycaprolactone, poly(α-hydroxy
acids) and copolymers prepared from the monomers of
these polymers.

12. The particle of claim 6 wherein the
multifunctional compound is selected from the group
consisting of dextrins, pentaerythritol, glucaronic
acid, tartaric acid, mucic acid, citric acid,
benzene dicarboxylic acid, benzene tricarboxylic
acid, benzene tetracarboxylic acid and butane
diglycidyl ether.

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13. The particle of claim 6 comprising
molecules covalently bound to the surface of the
particle via reactive groups on the hydrophilic
polymer, wherein the molecules are selected from
the group consisting of biologically active
molecules, non-biologically active molecules which
can be detected, targeting molecules, and molecules
affecting the charge, lipophilicity or
hydrophilicity of the particle.

14. The particle of claim 13, wherein the
targeting molecule is selected from the group
consisting of compounds specifically reactive with
a cell surface component, antibodies and antibody
fragments.

15. The particle of claim 6 wherein the
diameter is less than one micron.

16. The particle of claim 6 wherein the
diameter is between one and 1000 microns.

17. The particle of claim 13 wherein the
detectable molecule is selected from the group
consisting of substances detectable by x-ray,
fluorescence, ultrasound, magnetic resonance
imaging and radioactivity.

18. The particle of claim 8, wherein the
poly(alkylene glycol) is poly(ethylene glycol).

19. The particle of claim 6 formed of a core
of a different material than the coblock polymer
coating.

20. A method for making a multiblock
copolymer by covalently linking a multifunctional
compound to one or more hydrophilic polymers and
one or more hydrophobic bioerodible polymers,
wherein the number of polymer blocks is at least
three.

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21. The method of claim 20 further comprising
forming a particle with a diameter between 50 nm
and 1000 μm of the coblock polymer or coating a
particle with a diameter between 50 nm and 1000 μm
with the coblock polymer.

22. The method of claim 21 further comprising
incorporating a substance in the particle.

23. The method of claim 22 wherein the
substance is a biologically active substance
selected from the group consisting of peptides,
proteins, carbohydrates, nucleic acids, lipids,
polysacccarides, combinations thereof, and
synthetic inorganic or organic molecules that cause
a biological effect when administered *in vivo* to an
animal.

- 24. The method of claim 20 wherein the
hydrophilic polymer is selected from the group
consisting of polyalkylene glycols, polyvinyl
alcohols, polypyrrolidones, poly(amino acids),
oxidized cellulose and dextrans.

25. The method of claim 24 wherein the
poly(amino acid) is selected from the group
consisting of gelatin, fibrinogen and albumin
fragments.

26. The method of claim 20 wherein the
hydrophobic polymer is selected from the group
consisting of polyphosphazenes, polyphosphate
esters, polyanhydrides, polyhydroxybutyric acid,
polyorthoesters, polycaprolactone, poly(α-hydroxy
acids) and copolymers prepared from the monomers of
these polymers.

27. The method of claim 20 wherein the
multifunctional compound is selected from the group
consisting of dextrins, pentaerythritol, glucaronic
acid, tartaric acid, mucic acid, citric acid,
benzene dicarboxylic acid, benzene tricarboxylic

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acid, benzene tetracarboxylic acid and butane
diglycidyl ether.

28. The method of claim 21 further comprising
covalently binding to the surface of the particle
via the terminal hydroxyl group of the
poly(alkylene glycol) molecules selected from the
group consisting of biologically active molecules,
non-biologically active molecules which can be
detected, targeting molecules, and molecules
affecting the charge, lipophilicity or
hydrophilicity of the particle.

29. The method of claim 28 further comprising
targeting the particle for delivery to a specific
cell type by attaching to the surface of the
particle a targeting molecule selected from the
group consisting of compounds specifically reactive
with a cell surface component, antibodies and
antibody fragments.

30. The method of claim 28 wherein the
molecule is a substance detectable by x-ray,
fluorescence, magnetic resonance imaging,
ultrasound or radioactivity.

31. A method for delivering a substance to a
patient comprising administering to the patient a
particle having a diameter of between 50 nm and
1000 μm formed of or coated with a multiblock
copolymer formed by covalently linking a
multifunctional compound with one or more
hydrophilic polymers and one or more hydrophobic
bioerodible polymers to form a block copolymer
including at least three polymer blocks.

32. The method of claim 31 wherein the
substance to be delivered is a biologically active
substance selected from the group consisting of
peptides, proteins, carbohydrates, nucleic acids,
lipids, polysaccharides, combinations thereof, and

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synthetic inorganic or organic molecules that cause
a biological effect when administered to an animal.

33.   The method of claim 31 wherein the
hydrophilic polymer is selected from the group
consisting of polyalkylene glycols, polyvinyl
alcohols, polypyrrolidones, poly(amino acids),
oxidized cellulose and dextrans.

34.   The method of claim 33 wherein the
polyalkylene glycol is selected from the group
consisting of polybutylene glycol, polyethylene
glycol and copolymers of polyoxyethylene and
polyoxypropylene.

35.   The method of claim 31 wherein the
hydrophobic polymer is selected from the group
consisting of polyphosphazenes, polyphosphate
esters, polyanhydrides, polyhydroxybutyric acid,
polyorthoesters, polycaprolactone, poly(α-hydroxy
acids) and copolymers prepared from the monomers of
these polymers.

36.   The method of claim 31 wherein the
multifunctional compound is selected from the group
consisting of dextrins, pentaerythritol, glucaronic
acid, tartaric acid, mucic acid, citric acid,
benzene dicarboxylic acid, benzene tricarboxylic
acid, benzene tetracarboxylic acid and butane
diglycidyl ether.

37.   The method of claim 22 further comprising
delivering a substance to a patient comprising
administering to the particle to the patient,
wherein the substance to be delivered is a
biologically active substance selected from the
group consisting of peptides, proteins,
carbohydrates, nucleic acids, lipids,
polysacccarides, combinations thereof, and
synthetic inorganic or organic molecules that cause
a biological effect when administered *in vivo* to an
animal.

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38.   The method of claim 30 further comprising
delivering a substance to a patient by
administering to the particle to the patient,
wherein the molecule is selected from the group
consisting of substances detectable by x-ray,
fluorescence, ultrasound, magnetic resonance
imaging and radioactivity.

39.   The multiblock copolymers of claim 1,
further comprising a linear copolymer in a ration
of between 1 and 99% by weight of the total polymer
mixture.

40.   The particles of claim 6, further
comprising a linear copolymer in a ratio of between
1 and 99% by weight of the total polymer mixture.

41.   The method of claim 20, further
comprising mixing a linear polymer with the non-
linear multiblock copolymer in a ratio of between 1
and 99% by weight.

42.   The method of claim 33, further
comprising mixing a linear polymer with the non-
linear multiblock copolymer in a ratio of between 1
and 99% by weight.

FIG. 1a

OTHER STRUCTURES

MICROENCAPSULATION

FIG. 1b

18
LIGAND

18
LIGAND

FIG. 1c

PEG
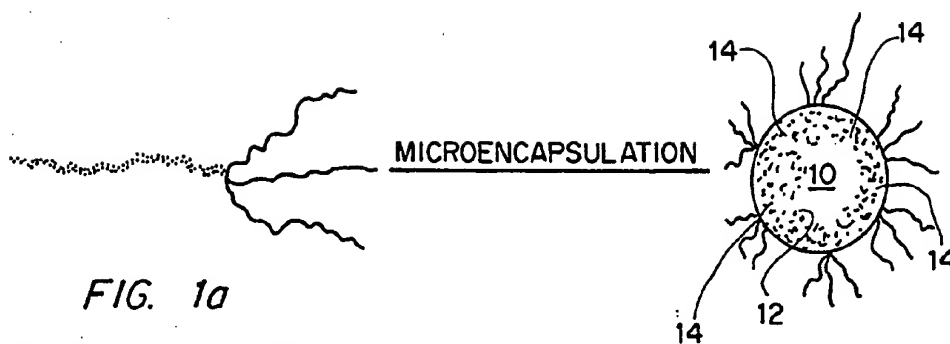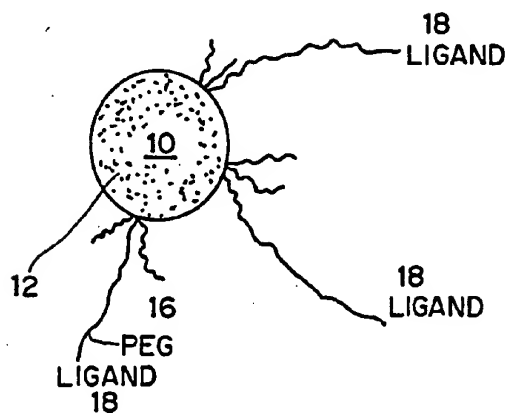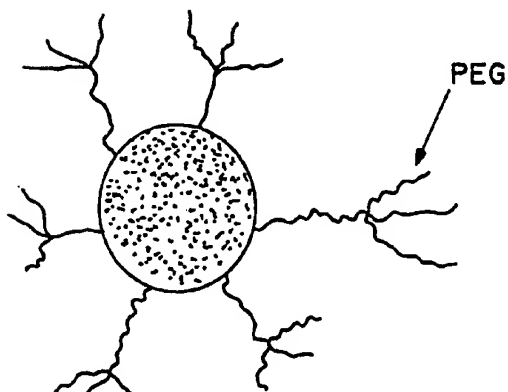
FIG. 2a

Compound   Schematic rep.

A.

B.

H.

FIG. 2b

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FIG. 2c

G.

$$CH_3-(-O-CH_2-CH_2-)_n-O-CH_2-CH-CH_2$$

with ester groups $O-(-C-CH-O-)_n-$ (C=O, CH_3) branches

PEG—⌐PLA
     ⌐PLA (PEG branching to two PLA)

FIG. 2d

I

$$CH_3-(-O-CH_2-CH_2-)_m-NH-C(=O)-\cdots-C(=O)-NH-(-CH_2-CH_2-O-)_m-CH_3$$

$$CH_3-(-O-CH-C-)_n-O-CH_2-CH_2-O-C-\cdots-C-O-CH_2-CH_2-O-(-C-CH-O-)_n-CH_3$$

(benzene ring core with four substituents)

PEG⌐ ⌐PEG
PLA⌐ ⌐PLA

OR

I-1.

$$CH_3-(-O-CH_2-CH_2-)_m-NH-C(=O)-\cdots-C(=O)-NH-(-CH_2-CH_2-O-)_m-CH_3$$

$$CH_3-C-(-O-C-R-C-)-O-C-\cdots-C-O-[-C-(CH_2)_x-C-O-]_n-C-CH_3$$

(benzene ring core with four substituents)

PEG⌐ ⌐PEG
PSA⌐ ⌐PSA
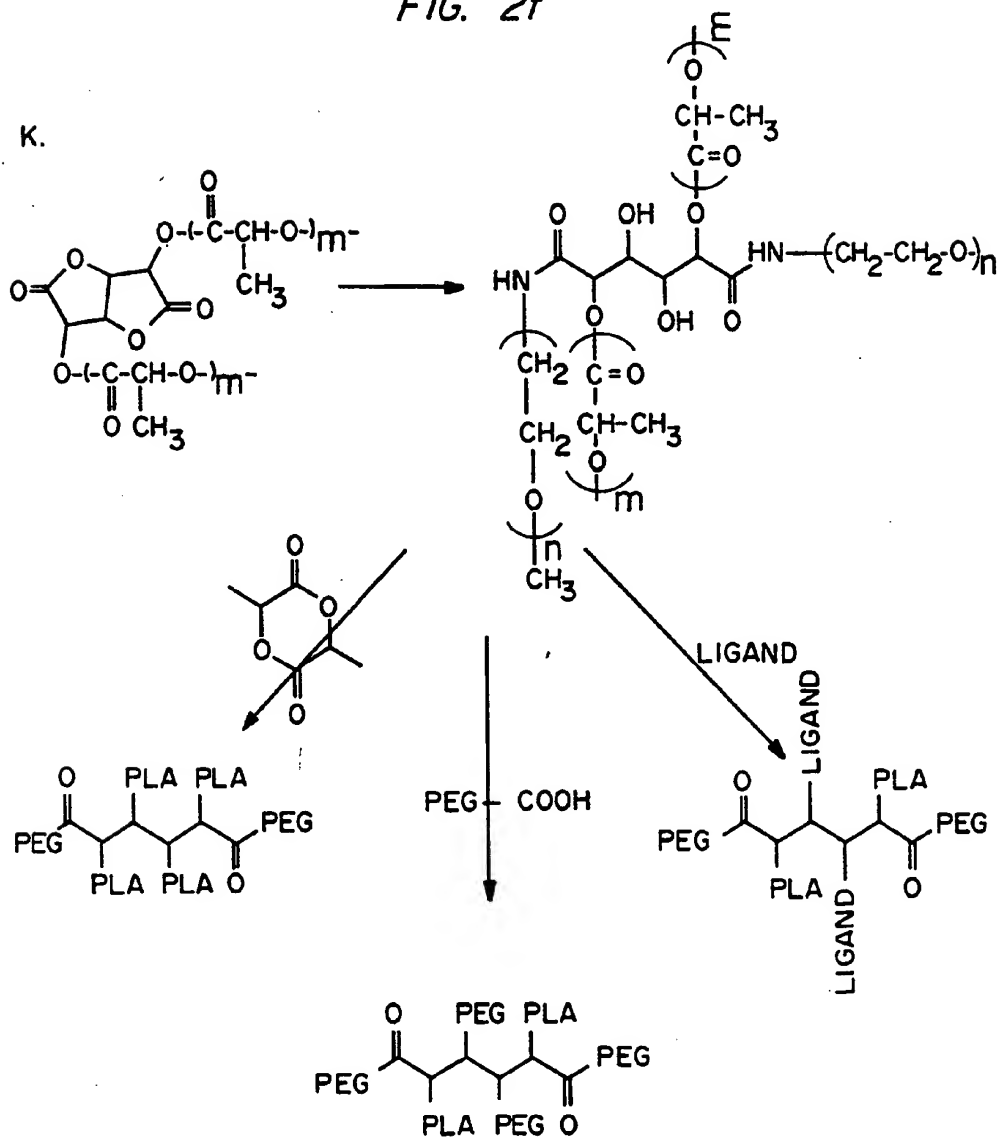
5/7

FIG. 2e

J.

FIG. 2f

K.

FIG. 2g

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FIG. 2h

N.

$$
\begin{array}{c}
\text{O} \\
\parallel \\
\text{C-NH-CH}_2\text{-CH}_2\text{-N=C-Dextrane} \\
\mid \\
\text{CH}_2 \\
\mid \\
\text{PLA-O-C-CO-NH-CH}_2\text{-CH}_2\text{-N=C-Dextrane} \\
\mid \\
\text{CH}_2 \\
\mid \\
\text{C-NH-CH}_2\text{-CH}_2\text{-N=C-Dextrane} \\
\parallel \\
\text{O}
\end{array}
$$

PLA —┬— Dextrane
    ├— Dextrane
    └— Dextrane

O-1.

$$
\begin{array}{c}
\text{C=N-CH}_2\text{-CH}_2\text{-N=Dextrane} \\
\mid \\
\text{PLA-O-CH} \\
\mid \\
\text{(CH}_2\text{)}_3 \\
\mid \\
\text{C=N-CH}_2\text{-CH}_2\text{-N=Dextrane}
\end{array}
$$

PLA —┬— Dextrane
    └— Dextrane

FIG. 2i

O.

$$
\begin{array}{c}
\text{C=N-(-CH}_2\text{-CH}_2\text{-O-)}_n\text{-CH}_3 \\
\mid \\
\text{PLA-O-CH} \\
\mid \\
\text{CH}_2 \\
\mid \\
\text{CH}_2 \\
\mid \\
\text{CH}_2 \\
\mid \\
\text{C=N-(-CH}_2\text{-CH}_2\text{-O-)}_n\text{-Ligand or CH}_3
\end{array}
$$

PLA —┬— PEG
    └— PEG

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6   C08G81/00   C08J3/12   C08G63/664   A61K9/51   A61K9/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6   C08G   A61K   C08J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	EP,A,0 552 802 (EASTMAN KODAK COMPANY) 28 July 1993 see claims 1,3-7,9,10 see page 2, line 6 - line 10 see examples see page 2, line 41 - page 4, line 35	1
A	EP,A,0 295 055 (YISSUM R&D COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM) 14 December 1988 see claims 1,16,20,21	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 November 1994	15. 12. 94

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Niaounakis, M |

Form PCT/ISA/210 (second sheet) (July 1992)

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EP-A-0552802	28-07-93	CA-A-	2087125	24-07-93
		JP-A-	5295120	09-11-93
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		US-A-	4826945	02-05-89